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Serial Changes During Acute Cardiac Allograft Rejection: Quantitative Ultrasound Tissue Analysis Versus Myocardial Histologic Findings

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Objectives. **The aim of this study was to assess 1) whether quantitative ultrasound tissue analysis by serial measurements of myocardial echo amplitudes can detect and monitor the onset and degree of acute cardiac rejection, as well as its resolution of acute rejection during treatment, and 2) whether changes in myocardial echo amplitudes are modified by repeat additional rejection episodes.**

Background. **Previous experimental studies, all involving heterotopic heart transplantation, have consistently shown reproducible alterations in myocardial echo amplitude during acute rejection episodes untreated by immunosuppressive agents.**

Methods. **Two-dimensional echocardiographic long-axis views were obtained daily under strict standardization in 12 dogs after heterotopic cervical heart transplantation (mean survival time 16.1 days) and digitized into a 256 × 256 × 8 matrix. Myocardial echo amplitudes were analyzed by gray level histogram statistics in regions of interest (45 × 12 pixels) within the proximal septum and posterior wall and correlated with the results of daily transmural myocardial biopsies. Maintenance immunosuppressive therapy consisted of cyclosporine, azathioprine and steroids. Additive steroids were given during acute cardiac rejection.**

Results. **All dogs experienced at least one moderate or severe episode of acute cardiac rejection. Successful resolution and repeat acute rejection were observed in three dogs. On 65 days, the left ventricular biopsy specimens showed no evidence of acute rejection. Mild acute rejection was present on 36, moderate on 29 and severe rejection on 40 days. End-diastolic mean (± SD) gray level increased progressively from 100.7 ± 20.4 for no acute cardiac rejection to 113.8 ± 23.1 for mild rejection (p = NS vs. no rejection) to 126.0 ± 16.1 for moderate rejection ($p < 0.01$) and to 136.3 ± 12.6 for severe rejection ($p < 0.01$). In each individual dog, a correlation between daily measurements of mean gray levels and histologic cardiac rejection grades was found (r_{mean} = 0.80 ± 0.14 [range 0.57 to 0.97], n = 12). In three dogs with transient complete histologic resolution of acute cardiac rejection, mean gray level did not return to values before rejection (108.0 ± 15.4 vs. 87.2 ± 8.4). The subsequent second episode of rejection was characterized by higher gray level values than those associated with the first rejection episode (141.3 ± 14.4 vs. 124.3 ± 20.9).**

Conclusions. **Acute cardiac rejection is associated with a progressive increase in mean gray level. Changes in myocardial echo amplitudes in individuals may thus prove a useful tool for the noninvasive detection and monitoring of acute rejection. Increased mean gray level values after resolution of rejection may indicate persistent structural tissue abnormalities after rejection and demonstrate the need to define new baseline values after histologic resolution of an acute rejection episode.**

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The early recognition of acute rejection episodes in cardiac allograft recipients remains a major challenge (1). The standard method used to identify rejection is endomyocardial biopsy, but this technique has important inherent limitations (2–5). Therefore, a noninvasive and highly sensitive method for the detection of cardiac allograft rejection remains a desirable goal.

Ultrasound tissue analysis is a relatively new approach that can identify and characterize myocardial changes based on the analysis of interactions between ultrasound and tissue (6,7). Several previous experimental animal studies, all involving heterotopic heart transplantation, have consistently shown reproducible alterations in myocardial echo amplitude during acute rejection episodes untreated by immunosuppressive agents (8–10). However, it has not been established whether these alterations could be used as a reliable diagnostic tool for detecting the onset of rejection and monitoring its resolution after intensified immunosuppressive therapy. In addition, no studies have compared daily histologic changes and myocardial echo amplitudes during the onset and resolution of rejection. A recent study

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in humans (11) has demonstrated that rejection-related changes occur in the acoustic properties of the myocardium when assessed by the cyclic variation of integrated ultrasound backscatter. However, the time intervals between biopsies in that investigation were long, because daily histologic follow-up studies cannot be performed in a clinical setting.

This experimental study was devised to determine whether quantitative ultrasound tissue analysis can detect and monitor acute cardiac rejection by serial evaluations of myocardial echo amplitudes. Histologic alterations of the myocardium were to be assessed by daily transmural myocardial biopsies and correlated with the echocardiographic findings. A maintenance immunosuppressive regimen and a protocol for the treatment of rejection episodes similar to that used in transplant patients was chosen to achieve a situation comparable to that in human allografts. Changes in myocardial echo amplitude were to be analyzed for their reliability to determine the histologic onset and degree of acute cardiac rejection and its resolution during treatment. Finally, we examined whether myocardial echo amplitude is modified by a repeat episode of acute cardiac rejection.

Methods

We chose for study a cervical heart transplant model that was first described by Carrel and Guthrie (12) and modified by Mann et al. (13). This model provides access for transmural biopsies of both ventricles, as well as an excellent echocardiographic window for acquisition of high quality ultrasound images, because there is little intervening tissue between the heart and transducer.

Animal preparation. Twenty healthy mongrel dogs (20 to 31 kg) were selected as recipients of cardiac allografts from smaller-sized donor mongrels (12 to 26 kg). All animals were anesthetized with intravenous methohexital (5 mg/kg body weight) and after endotracheal intubation were ventilated with a gas mixture of nitrous oxide and oxygen (2:1), as well as halothane (0.6 to 2%). Pancuronium bromide (0.2 mg/kg intravenously), was given for muscle relaxation. A femoral artery cannula (5F Seldinger) was inserted to monitor blood pressure and arterial blood gases. Heart rate was recorded by electrocardiographic (ECG) limb lead tracings.

Donor preparation. The heart was approached through a midsternal thoracotomy. The azygous vein was ligated and loose ties were placed around the aorta, the superior and inferior venae cavae, the brachiocephalic artery and the pulmonary veins. After administration of heparin (5,000 U), the brachiocephalic artery was ligated distally, divided and cannulated proximally for subsequent perfusion with hyperkalemic crystalloid cardioplegic solution (Brettschneider, 4°C). In addition, topical cooling was applied (4°C saline solution). After division of the great vessels, the donor heart was excised, weighed and maintained in 4°C saline solution.

Receiver preparation and transplant anastomosis. Each recipient dog was placed on the left side and the neck was prepared to expose the right common carotid artery and external jugular vein. The donor heart was anastomosed to the neck vessels using an end to side technique between the pulmonary artery and right jugular vein, as well as between the aorta and common carotid artery with continuous 6-0 polypropylene sutures. After coronary perfusion was started, regular sinus rhythm was established either spontaneously or after electrical cardioversion. Total ischemic time averaged 88.9 ± 25.6 min (range 51 to 127). When a stable hemodynamic status was achieved, the donor heart was placed in the recipient's neck pouch and skin flaps were closed.

With this technique, blood flowed into the donor aorta and coronary vessels from the recipient's carotid artery under systemic pressure. The right ventricle was filled from coronary venous blood flow, which returned to the recipient's circulation by way of the pulmonary artery. Because no artificial atrial septal defect was created, the left ventricle was filled only from thebesian veins and some retrograde flow across the aortic valve. Retrograde ventriculography and coronary angiography (n = 4) showed sufficient pressure (peak systole 101.5 ± 12.0 mm Hg, peak end-diastole 6.5 ± 3.5 mm Hg) and oxygen saturation (88.5 ± 3.5%). Coronary perfusion was normal.

Experimental protocol. All dogs routinely received carbapeneme (250 mg two times daily) for the 1st 5 postoperative days and benzylpenicillin (0.5×10^6 immunizing units every other day) on the subsequent days. In addition, aspirin (100 mg/day) and an H_2-blocking agent (25 mg/day) were given.

The immunosuppressive protocol consisted of cyclosporine, azathioprine (1 mg/kg per day) and prednisolone (0.2 mg/kg per day). Cyclosporine levels were measured twice a week and the actual dosage of cyclosporine was adapted to maintain whole blood levels between 400 to 800 ng/ml (radioimmunoassay). During moderate or severe episodes of acute rejection, methylprednisolone (125 mg) was administered on 3 consecutive days.

Donors and recipients were not cross-matched for dog erythrocyte antigen (DEA) or dog lymphocyte antigen (DLA); thus, virtually all dogs were expected to develop acute allograft rejection. All animals received humane care in accordance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 80-23, revised 1978).

Biopsy specimen acquisition. All dogs were anesthetized with intravenous methohexital (5 mg/kg) and a phenothiazine derivative (1.2 mg/kg). Transmural biopsies were performed daily from postoperative day 2, using a rigid bioptome (Biopty-Cut 5F, Bard). Only one specimen/ventricle (mean sample length 6 mm) was taken; therefore, a high sampling error was knowingly accepted (14). For each procedure the skin suture line was partially reopened, and a left ventricular

Table 1. Histologic Biopsy Grading System

Rejection Category and Score	Criteria
None	
0	No evidence of rejection
0.5	Isolated lymphocytes
Mild	
1	Focal perivascular or interstitial infiltrate
1.5	As 1+ myocyte encroachment without necrosis
Moderate	
2	Multifocal infiltrates and myocyte damage
Borderline severe	
2.5	Diffuse aggressive inflammatory infiltrate and myocyte damage
Severe	
3	As 2.5+ myocyte necrosis and hemorrhage

biopsy specimen was obtained from a different site each day. A right ventricular biopsy was also performed to assess the regional distribution of the rejection process in both ventricles. All biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. If a pericardial effusion was present, this was eliminated before closing the skin to avoid its influence on myocardial acoustic properties during the following echocardiographic examination.

Ultrasound data acquisition. Two-dimensional echocardiograms were recorded with a commercially available electronic sector scanner (Toshiba SSH 65A, Toshiba Medical Systems Europe) equipped with a short focus phased array transducer (Toshiba PSB 50 SS, 5 MHz center frequency, 3.5-cm focal distance, 3-cm focal length). A conventional 90° sector image with 100 scan lines was formed at a rate of 48 Hz. For each dog, gain settings and gain compensation profiles were adjusted at the time of the first examination to achieve optimal visualization of the ventricular walls with uniform myocardial brightness throughout the echocardiogram. Gain settings were then kept constant throughout the entire study period. Each dog was examined daily in a left lateral decubitus position by the same physician, beginning immediately after the operation. The transducer was positioned with firm pressure on the overlying skin flap. Pressure was then reduced, until the visible skin impression disappeared. The transducer position was adjusted such as to obtain two-dimensional long-axis views of the left ventricle corresponding to the images of the previous day. Echocardiographic variables such as wall thickness, chamber size and fractional shortening were measured from M-mode recordings of the left ventricle. Two-dimensional echocardiographic images were recorded on videotape (JVC BR 6400 TR) for later analysis.

Histopathologic examination. All biopsy specimens were stained with hematoxylin-eosin and evaluated for the degree of rejection using a modification of the Billingham criteria (14,15, Table 1). Histologic evaluations were performed on the same day by consensus of two experienced pathologists. Cardiac allografts were excised when electrical and mechanical activity had stopped. After excision, heart weight and any gross morphologic changes were recorded and full-thickness tissue specimens were obtained for histologic analysis.

Image processing and analysis. Three consecutive cardiac cycles of the left parasternal long-axis view were digitized from the videotape into an image processing computer (Mipron, Kontron Electronics). Gray level resolution was 8 bits in a 256 × 256 pixel matrix. Image memory rate was 25 frames/s.

End-diastolic images were selected at the onset of the Q wave in the simultaneously recorded allograft electrocardiogram. Regions of interest measuring 45 × 12 pixels; were defined within the proximal parts of the ventricular septum and posterior left ventricular wall, excluding specular reflections from the endocardium and epicardium. Care was taken to keep the position of the region of interest constant throughout the examination sequence of each individual dog using the mitral anulus and the posterior papillary muscle as internal landmarks. Lateral offset from the center line of the scan was less than 10° within the region of interest to minimize azimuth-dependent gray level histogram changes (16). The histologic results of the biopsies were not known to the two investigators who performed the echocardiographic evaluation. Interobserver variability was evaluated by two independent observers who chose regions of interest on the same day. Intraobserver variability was evaluated by one observer on subsequent days. Reproducibility analyses were performed for mean gray levels within the posterior wall in six dogs. Myocardial echo amplitudes within each region of interest were analyzed by gray level histogram statistics as previously described (17–19). Extracted variables were 1) mean gray level, which describes the average gray value distribution within the region of interest, 2) standard deviation, which expresses the spreading of the distribution, 3) skewness, which measures the asymmetry of the shape of the distribution and 4) kurtosis, which calculates the "peakedness" of the distribution relative to the length and size of its tails.

Statistical analysis. All data are expressed as mean value ± SD. In each dog, mean values of M-mode variables and gray level histogram statistics were calculated during episodes of no, mild, moderate and severe rejection, as proved by biopsy. Mean values of all dogs were compared for the different rejection grades using analysis of variance, the Scheffé F test for statistical significance and a test for overall differences over time. Inter- and intraobserver variability was determined by linear regression analysis and by mean percent difference of the measurements. Gray level histogram statistics and M-mode variables were correlated with the daily histologic rejection grade in each individual dog using linear regression analysis. Before comparing correlation coefficients with analysis of variance, Fisher's transformation was performed.

Results

Allograft function and survival. Eight of the 20 allografts were excluded from analysis because of perioperative or early postoperative complications, defined as graft failure or recipient death during the 1st 4 days. In three dogs, a stable hemodynamic status was never established postoperatively. Additional reasons for exclusion were cardiac tamponade (two dogs), bleeding from the aortic anastomosis (one dog), gastrointestinal bleeding (one dog) and malignant arrhythmias (one dog).

Thus, data from 12 recipient dogs were evaluated. Mean postoperative heart rate of the transplanted hearts was 114.7 ± 10.4 beats/min (range 68 to 154). Mean survival time was 16.1 ± 7.2 days (range 6 to 28). Allograft failure occurred in eight dogs during the first acute rejection episode (mean survival time 12.6 ± 5.6 days). In four allografts, histologic resolution of the first rejection episode was achieved after treatment, and transplant failure occurred during a second severe rejection episode (mean survival time 23.0 ± 4.8 days).

Histopathologic findings. Satisfactory left ventricular biopsy samples were obtained in 170 of a total of 177 biopsies. Biopsy failure occurred on seven occasions because biopsy material was insufficient or the sample was acquired from a previous biopsy site. On 16 days biopsy could not be performed because of hemodynamic conditions in the transplanted heart.

On 65 days, the left ventricular biopsy specimen showed no evidence of acute rejection. Mild acute rejection was present on 36, moderate on 29 and severe rejection on 40 days. Each dog had at least one episode of moderate or severe rejection (severe rejection in nine dogs, moderate rejection in three). The mean time interval between the operation and the onset of the first rejection episode was 5.8 ± 3.3 days.

In the three dogs with complete resolution of rejection (return to biopsy grade 0), histologic evaluation showed edema and granulation tissue without cellular infiltrates; myocardial fibrosis was present in one of these dogs. Resolving rejection (lesser biopsy grade for several days) was found in one dog. The second rejection episode occurred 9.3 ± 1.0 days after cessation of rejection therapy in these four animals. Intermittent short-term decreases in biopsy grade occurred in several dogs, probably because of the high sampling error associated with taking only one biopsy sample/day (14), although regional differences in rejection severity may also have played a role (20). For the purpose of this study, we assumed that short-term decreases in histologic severity did not reflect true changes in the rejection process. A decrease in histologic severity by at least one biopsy grade for only 1 day was thus defined as a false negative result. During such episodes no additional rejection treatment was administered.

In 67.3% of biopsies, the left and right ventricles had a similar rejection grade. A higher degree of cardiac rejection was present in 18.2% of left ventricular and in 14.5% of the right ventricular biopsy specimens. The excised hearts showed an increase in wall thickness and ventricular mass. Mean donor heart weight increased from 145 ± 29 g (range 93 to 190) before implantation to 235 ± 55 g (range 150 to 307) after excision. Histologic examination revealed diffuse aggressive, inflammatory infiltrates of lymphocytes, eosinophils and neutrophils. Myocyte necrosis, edema, hemorrhage and vasculitis were seen in all wall segments.

Ultrasound findings. *M-mode variables of the left ventricle versus histologic findings.* Mean end-diastolic total wall thickness (septal plus posterior wall) increased from 18.4 ± 1.4 mm at no rejection, to 19.0 ± 2.0 mm at mild (p = NS vs. no rejection), 19.8 ± 1.9 mm at moderate (p = NS vs. no rejection) and 20.5 ± 1.7 mm at severe rejection ($p < 0.05$ vs. no rejection). No significant change in left ventricular end-diastolic diameter was found (27.7 ± 4.7 mm at no rejection, 27.6 ± 4.9 mm at mild, 28.2 ± 6.1 mm at moderate and 29.9 ± 6.9 mm at severe rejection).

There was a weak but statistically significant correlation between daily cardiac biopsy grades and M-mode mean total wall thickness in 10 of 12 dogs (r_{mean} = 0.55 ± 0.19 [range 0.41 to 0.81], n = 10). No correlation was found in two dogs, in which wall thickness was transiently increased postoperatively, possibly as a consequence of prolonged ischemic time. The mean correlation coefficient improved after elimination of days with a false negative biopsy result (r_{mean} = 0.64 ± 0.17, $p < 0.05$ [range 0.41 to 0.93], n = 12).

Changes in myocardial echo amplitude versus histologic findings. Mean septal gray level increased progressively from 100.7 ± 20.4 at no rejection, to 113.8 ± 23.1 (p = NS) at mild, 126.0 ± 16.1 ($p < 0.01$) at moderate and 136.3 ± 12.6 ($p < 0.01$) at severe rejection. Changes were similar in the posterior wall with 102.9 ± 30.8 at no, 108.9 ± 34.2 (p = NS) at mild, 118.1 ± 32.6 (p = NS) at moderate and 136.2 ± 32.4 ($p < 0.05$) at severe rejection. Figures 1 and 2 compare the echocardiographic and histologic appearance of normal and rejecting myocardium. The echocardiographic images were obtained under strictly standardized conditions. Myocardial edema and cellular infiltrates are accompanied by a visible increase in mean gray level and wall thickness. Other variables derived from the gray level histogram such as standard deviation, skewness and kurtosis showed no significant changes or trends during different rejection grades.

Reproducibility analyses demonstrated a mean interobserver correlation of r = 0.91 (SEE = 9.7, $p < 0.01$) and a mean intraobserver correlation of r = 0.96 (SEE = 5.8, $p < 0.01$). Mean percent differences in the interobserver and intraobserver measurements were 8% and 4%, respectively. Figure 3 demonstrates the sequential changes in mean gray level (bottom panel) in one individual dog, which parallel those of cardiac biopsy grading (top panel) with an increase occurring at the onset of the first and also the second rejection episode. Significant correlation coefficients be-

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July 1993:310–17

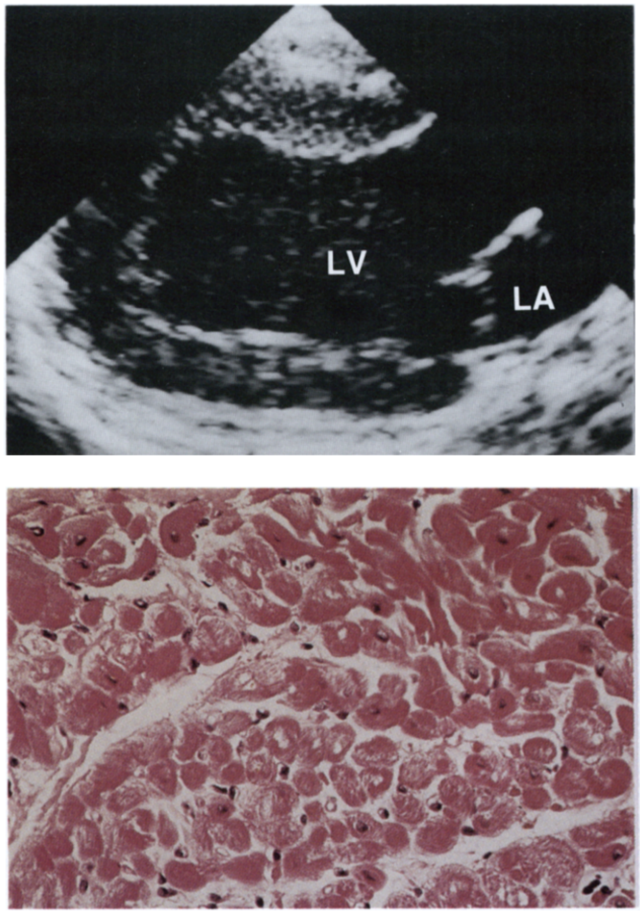

Figure 1. Echocardiographic long-axis cross section of cardiac allograft **(top panel)** and histologic section of transmural biopsy specimen from the left ventricular posterior wall **(lower panel)** on day 2 after transplantation (no acute rejection). Hematoxylin-eosin ×250, reduced by 35%. LA = left atrium; LV = left ventricle.

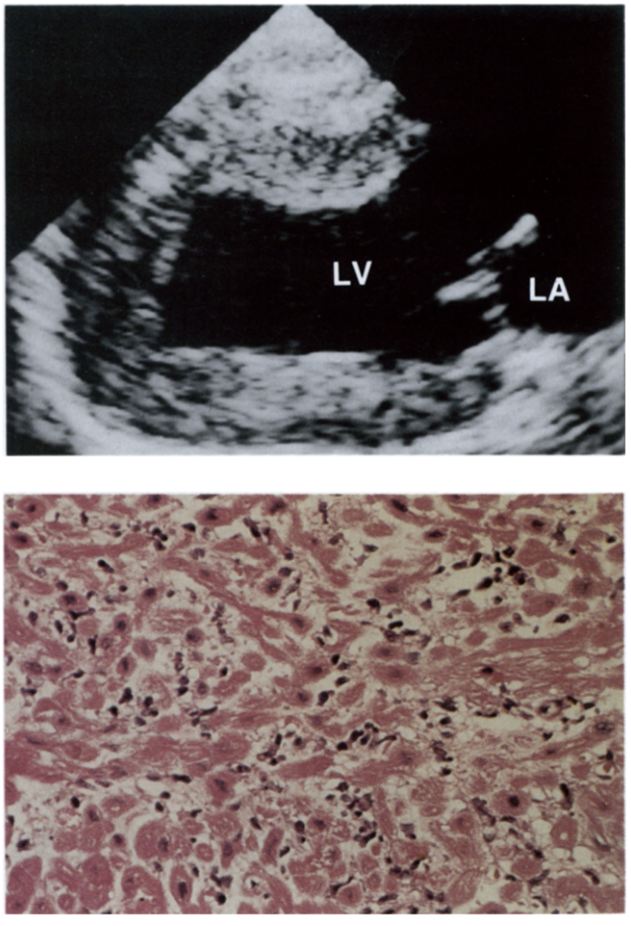

Figure 2. Echocardiographic long-axis cross section of cardiac allograft **(top panel)** on day 10 (moderate acute rejection) demonstrates a significant increase in myocardial mean gray level and wall thickness (same animal as in Fig. 1). The histologic section of transmural biopsy specimen obtained the same day **(lower panel)** shows multifocal cellular infiltrates and myocyte damage. Hematoxylin-eosin ×250, reduced by 35%. Abbreviations as in Figure 1.

tween rejection grade and mean gray level were found in 10 dogs (r_{mean} = 0.74 ± 0.14 [range 0.49 to 0.93], n = 10, Table 2). Dogs 16 and 18 showed no significant correlation, possibly as a consequence of multiple false negative biopsy results. After elimination of days with a false negative biopsy result, all 12 dogs showed significant correlation coefficients (r_{mean} = 0.80 ± 0.14 [range 0.57 to 0.97], n = 12, Table 2).

Separate analysis of the three dogs with a transient complete resolution of rejection showed a maximal increase in mean gray level during the first acute rejection episode in the septum and posterior wall from 87.2 ± 8.4 to 124.3 ± 20.9 and from 75.2 ± 22.8 to 106.4 ± 18.3, respectively. With resolved rejection, septal and posterior wall mean gray levels decreased only to 108.0 ± 15.4 and 93.6 ± 23.6, respectively. The second rejection episode was associated with even higher maximal mean gray levels (141.3 ± 14.4 and 136.8 ± 24.5). Figure 4 shows the changes in mean gray level, which differed between the first and second rejection episodes.

Discussion

Changes in myocardial echo amplitude and M-mode variables during allograft rejection. The histologic findings during the first cardiac allograft rejection were shown to parallel increases in mean gray level in all 12 dogs. Although mild acute rejection was associated with a slight increase in mean gray level, a significant rise was seen only during moderate to severe cardiac rejection.

These results correspond to those in a preliminary report by Dawkins et al. (10) in which color-coded two-dimensional echocardiography was used for the assessment of acute rejection. An increase in myocardial echo amplitude was also demonstrated by Chandrasekaran et al. (9), who showed a peak value on the 3rd postoperative day that was followed by a slight decrease over subsequent days. The authors attributed these changes to initial interstitial edema.

The exact relation between changes in the acoustic properties and underlying structural alterations of the myocar-

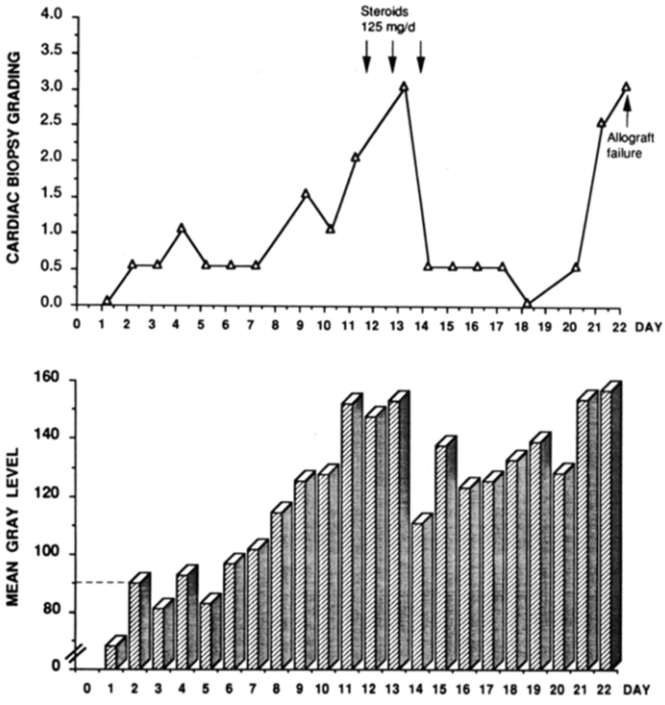

Figure 3. Sequential histologic and echocardiographic changes within the posterior wall in one of the dogs. Cardiac biopsy grading **(top panel)** and mean gray level **(bottom panel)** show a concomitant increase from days 1 to 13. During resolution of rejection after rejection therapy (days 14 to 18), mean gray level does not return to values before rejection. The second rejection episode (days 21 and 22) is accompanied by another increase of mean gray level. The transient decrease in the biopsy grade on day 10 probably corresponds to what was defined as a false negative biopsy result in this study.

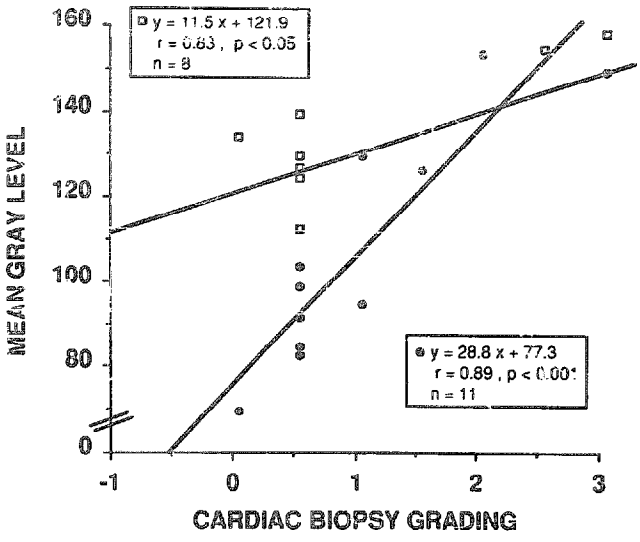

Figure 4. Scatterplot showing changes in mean gray level versus cardiac biopsy grading (same animal as in Fig. 3). Different slopes of rejection-induced mean gray level changes are observed during the first and second rejection episodes. (● = no acute rejection episode to first severe acute rejection episodes, days 1 to 13; □ = resolution of acute rejection to second rejection episode, days 14 to 22). Increased mean gray level baseline values (no acute rejection) are noted after the first rejection episode.

dium is still unclear. Myocardial backscatter is determined by a complex interaction between the ultrasound signal and individual scattering elements within the tissue. In this study, the concomitant progressive increase of myocardial echo amplitudes with increasing severity of the rejection process suggests that the development of myocardial edema, cellular infiltrates and, in more advanced stages, myocyte necrosis and hemorrhage may all have contributed to the observed changes. A recent experimental study in rats after heart transplantation (21) showed a progressive increase in myocardial water content and a decrease in myocardial tissue blood flow with increasing degrees of cardiac rejection. Both factors are known to influence myocardial ultrasound backscatter (22). A reduction in the coronary vascular response during acute rejection was reported by Nitenberg et al. (23). Such vascular alterations might be responsible for myocardial ischemia and focal necrosis, which could further contribute to a progressive increase in echo amplitudes during the rejection process (19,22,24,25).

Table 2. Correlation Coefficients (r values) Before and After Elimination of False Negative Biopsy Results (mean gray level vs. cardiac biopsy grading)

Dog No.	Interventricular Septum		Posterior Wall	
	Before	After	Before	After
1	0.85	0.85	0.63	0.82
2	0.58	0.74	0.34	0.43
4	0.69	0.95	0.69	0.93
5	0.83	0.89	0.48	0.77
7	0.93	0.94	0.73	0.99
10	0.76	0.74	0.76	0.71
11	0.74	0.74	0.87	0.87
12	0.49	0.57	0.60	0.66
16	0.32	0.60	0.57	0.94
18	−0.08	0.97	0.62	0.62
19	0.65	0.66	0.22	0.12
20	0.89	0.89	0.92	0.92

In an early M-mode echocardiographic study in human transplant patients, in which immunosuppression consisted of azathioprine and steroids, edema was considered the main reason for the marked increase in donor wall thickness and donor mass during cardiac rejection (26). In contrast, changes in wall thickness were subtle in the present investigation, in which immunosuppressive therapy included cyclosporine. The sensitivity of M-mode total wall thickness for the noninvasive detection of rejection was therefore limited. This observation is in accordance with previous experimental and clinical studies (27–31) performed during cyclosporine therapy and must be attributed to the specific effects of this immunosuppressive agent, which modifies the morphologic changes associated with the rejection process (32,33).

Changes in myocardial echo amplitude during resolved and repeated rejection. In the three dogs with histologically resolved acute rejection episodes, the increase in mean gray level did not return to values before rejection; biopsy specimens showed edema and granulation tissue without cellular infiltrates. Fibrosis was found in one dog. Thus, the elevated baseline mean gray levels could be explained by remaining edema and granulation tissue. In addition, the formation of collagen after acute rejection might lead to a persistent increase in myocardial echo amplitude (34–38). Corresponding changes could also have been responsible for the reduced cyclic variation in integrated ultrasound backscatter after resolution of rejection observed by others in human cardiac allografts (11).

The extent to which abnormalities during resolved rejection episodes represented irreversible or reversible myocardial changes remained unclear in this study, because all three dogs with such resolution had another severe rejection episode within 2 weeks. A recent investigation in humans (39) indicates that myocytes have a capacity for regeneration after myocyte injury due to allograft rejection. During the second acute rejection episode, higher echo intensities could have been caused by a higher degree of myocyte necrosis and hemorrhage or by persistent myocardial alterations due to the previous rejection episode. It is unlikely that myocardial fibrosis due to cyclosporine administration played a role, because this condition has been observed only during long-term treatment (15,40,41).

Potential implications for rejection diagnosis in humans. Our results suggest that close intraindividual monitoring of myocardial echo amplitudes measured from video images obtained under strictly standardized conditions might prove a useful additional means of noninvasive diagnosis and monitoring of cardiac allograft rejection in humans. Previous studies have shown that no single echocardiographic variable facilitates the diagnosis of acute rejection with satisfactory sensitivity and specificity (30,31), possibly because allograft rejection may preferentially involve either ventricle and the severity of rejection may vary at different sites (20).

Remarkably, rejection-induced changes in myocardial acoustic properties were detected and quantified using a commercial electronic sector scanner. Without additional equipment, this method could thus be applied in the clinical setting. It is possible that analysis of the unprocessed radiofrequency signal would provide a more standardized and more sensitive diagnostic tool, which might also allow detection of more subtle structural changes of the heart muscle and thus, perhaps, facilitate the diagnosis of mild rejection episodes.

Limitations of the study. In this study, myocardial acoustic properties had to be derived from video data, because examinations were performed in an operating room without direct online data tranfer to an image processing computer. Because only video images could be acquired, the data were influenced by logarithmic amplification, demodulation and scan conversion. Variables derived from radiofrequency signals, such as integrated backscatter, would have been preferable because they represent a more robust and sensitive diagnostic tool. However, in our study, intraindividual echocardiographic changes measured from video data using strictly standardized examination conditions still showed significant changes of myocardial composition during rejection. One further limitation concerns our use of a cervical heterotopic transplant model because of its uncomplicated access for transmural biopsies. However, the hemodynamic conditions in this model are not comparable to those of an orthotopic transplant because of reduced diastolic filling. Therefore, cyclic gray level changes, which are related to myocardial function, could not have been used as a diagnostic tool (42,43). However, changes in end-diastolic echo intensity in individual dogs should not have been affected by the abnormal hemodynamic situation. Because there was less intervening tissue between the transducer and heart in a cervical transplant model, high quality echocardiograms could be obtained. Applicability of this diagnostic approach in studies performed through the chest wall has yet to be tested.

Because only one biopsy specimen per ventricle was taken, the expected sampling error was much higher than that associated with the standard endomyocardial biopsy technique, in which sampling error is reduced to ≈2% because four to six myocardial pieces are taken (14). In the present study, about 13% of the biopsy specimens fulfilled our criteria for a false negative result. The true sampling error might have been even higher because of an asymmetric pattern of rejection, as indicated by a different rejection grade for the right versus the left ventricle. Similarly, the diagnosis of fibrosis in resolved rejection may have been limited by the small number of biopsies/day. A final limitation concerns biopsy and ultrasound sampling sites. Whereas the daily biopsy samples had to be obtained from different areas of the left ventricular myocardium, regions evaluated for myocardial echo amplitudes were kept constant. Despite the sampling discrepancy a high overall correlation between echocardiographic and histologic results was found. This sampling discrepancy was knowingly accepted to avoid the influence of regional bleeding, swelling or scarring due to previous biopsies on myocardial acoustic properties.

Conclusions. In this investigation, the histologic changes induced by acute rejection were probably comparable to those occurring in human allograft recipients, because basic immunosuppressive and rejection therapy corresponded to that used in patients. At least under the ideal conditions of this animal experiment, intraindividual changes in myocardial echo amplitude proved useful for the noninvasive detection and monitoring of acute cardiac rejection. Thus, serial measurements of mean gray level from video images obtained under strictly standardized conditions may also prove useful for the postoperative monitoring of patients after heart transplantation. To maintain diagnostic accuracy, new

baseline measurements should be established after each successfully treated rejection episode.

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